Certificate of Facsimile Transmission

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below:

By:_____

Date:_____

Atty. Docket No.: O-98394 US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

MITTENBURG ET AL.

Serial Number: 09/744,282 Group Art Unit: 1644

Filed: January 22, 2001 Examiner: Huynh, P.

For: USE OF gp-39 IN IMMUNE DISEASES

Dear Sir:

DECLARATION

1. I, Andrea van Elsas, hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 17 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the captioned application or any patent issued thereon.

I declare as follows:

2. I am a Senior Research Scientists at Akzo Nobel N.V. (Organon) and have a Ph.D. in Biology. My primary job responsibilities include target discovery for

Rheumatoid Arthritis Drug Development. I am familiar with the contents of U.S. Patent Application No. 09/744,282.

3. To begin, I would like to define immunological tolerance, as it is understood in the art: Immunological tolerance - the art of functionally discriminating between 'self' and 'non-self.' Tolerance induction through the mucosal route is a naturally occurring immunological phenomenon that prevents harmful inflammatory responses to ingested (food components) or inhaled environmental, predominantly non-dangerous, antigens. The mucosae of the gastrointestinal tract are easily accessible for the induction of immunological tolerance which can be exploited in the antigen-specific treatment of autoimmune disease, a situation in which tolerance has inadvertantly been broken. One of the mechanisms by which mucosal administration of antigen may effectuate the establishment of immunological tolerance is by the induction of active suppression exerted by modulatory or regulatory T cells.

4. It has been shown that murine type II collagen arthritis is efficiently suppressed by intranasal administration of a non-related human cartilage antigen e.g. Human Cartilage (HC) gp-39 using a semi-therapeutic protocol (Joosten). In this study, both arthritis incidence and severity were significantly suppressed. Histological analysis of knee and ankle joints proved the absence of an abundant inflammatory process: almost no infiltrating cells and no proliferating synovial tissue were observed. Most interestingly, bone erosions and cartilage destruction were completely prevented by HC gp-39 treatment. This may have been caused by an effect of regulatory T cells on anti-CII antibody responses or could be the result of a direct or indirect suppression of the

inflammatory process in the joints, for instance through the local release of suppressive cytokines from infiltrating T cells. The results have demonstrated that the intranasal treatment with HC gp-39 triggered modulatory or regulatory mechanisms that interfered with the expression of disease in murine collagen induced arthritis. Since removal of nose-draining lymph nodes before the intranasal application of HC gp-39 was shown to abrogate delayed-type hypersensitivity reactions, the therapeutic mechanism most likely involved active immune cell regulation (Wolvers). In one potential scenario, intranasal application of HC gp39 in murine collagen-induced arthritis induced HC gp39-reactive regulatory or modulatory T cells that effectuated cross-tolerance in the affected joints.

5. Rheumatoid arthritis (RA) is believed to be an (auto)immune disease in which immunological tolerance to components resident in articular joints may be broken. RA is characterized by a chronic inflammatory infiltration of the synovial membrane which is associated with destruction of cartilage and bone. The disease seems to be driven by a tissue specific inflammatory attack on peripheral, cartilaginous joints. The association between RA and certain alleles of the DRB1 locus suggests that one or more joint specific antigens may be recognized by HLA class II-restricted T-cells, which in turn mediate or perpetuate the inflammatory process. In the case disease-inducing antigens or antigens involved further down the road in the pathogenesis of the disease are known, antigen specific immunological tolerance may be exploited therapeutically to regain control via the induction of modulatory or regulatory processes. One could envisage intervention in an antigen-specific immune response with the native antigen itself, or with the use of one or more immunodominant epitopes. Furthermore, one could make use of HC gp-39 – as a potentially cross-tolerance inducing protein – to reinforce modulator or

regulatory processes, since HC gp-39 is expressed in the synovium of patients with RA (Baeten). In such a situation, induction of modulatory or regulatory T-cells to control immune diseases, combined with the expression of HC gp-39 by cells of the monocyte/macrophage lineage at the site of inflammation (e.g. the synovium) resulting in (re)activation of the modulatory or regulatory phenotype of these cells, would be sufficient to realize intervention with HC gp-39 in the immune disease.

6. Evidence indicates that expression of HC gp-39 is related to monocyte to macrophage maturation (Krause). In contrast to many other monocyte/macrophage markers, its expression is absent in normal monocytes and strongly induced during late stages of human macrophage differentiation (Rehli). Other cell types that express HC gp-39 include chondrocytes and synovial cells (Hakala) and neutrophils (Volck), the latter cell type, like macrophages, being heavily involved in inflammatory processes. As a reflection of the involvement of HC gp-39 producing cells in immune disease conditions, elevated Human Cartilage glycoprotein-39 plasma levels have been detected for instance in patients with rheumatoid arthritis (Johansen), and in patients with other inflammatory conditions like osteoarthritis (OA), systemic lupus erythemathosus (SLE), and inflammatory bowel disease (IBD) (Vos). Furthermore, strong expression of HC gp-39 has been detected in lesion macrophages of atherosclerosis specimens (Boot). In summary, there is ample evidence for the expression of HC gp-39 in multiple (auto)immune conditions and it is clear that tissues that contain monocytes that mature to macrophages and/or neutrophilic granulocytes do contain HC gp-39 protein. Thus, mucosal application of HC gp-39 resulting in the activation or reactivation of modulatory or regulatory T cells could be expected to exert immune regulation in all conditions in

which cells of the monocyte/macrophage lineage, or neutrophilic granulocytes are present within the inflammatory lesion.

7. Such conditions include, but are not limited to, diseases like Graves' disease, primary glomerulonephritis, osteoarthritis, juvenile arthritis, Sjögren's disease, myasthenia gravis, rheumatoid arthritis, Addison's disease, primary biliary sclerosis, uveitis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis or diabetes. For instance, data have shown that there is a relative overexpression of macrophage-derived cytokines in orbital adipose tissue from patients with Graves' ophthalmopathy (Kumar), implicating an important role for macrophages in the inflammatory lesion in this disease. Macrophages, their activation products and the regulation of cell survival (e.g. through apoptosis) are suggested to play an important role in glomerulonephritis (D'Souza), osteoarthritis (Young), juvenile idiopathic arthritis (Ramanan), Sjögrens disease (Bredberg), myastenia gravis (Shi), rheumatoid arthritis (Mulherin), liver cirrhosis (Alric), uveitis (Pouvreau), systemic lupus erythematosus (Ren), inflammatory bowel disease (Klebl), multiple sclerosis (Bruck) or diabetes (Kusterer). The principle that HC gp-39 is expressed by macrophages on their way to full maturation (Krause, Rehli), in combination with the potential that HC gp-39 induces the activation or reactivation of modulatory or regulatory cells at the site of inflammation (Joosten, Wolvers), implies that HC gp-39 based immune intervention can be used in any (auto) immune condition. This intervention can be performed with the use of HC gp-39 protein containing a number of epitopes recognized by the immune system, or with the use of one or more selected peptides recognized by the immune system in various autoimmune conditions (Verheijden, Vos).

8. Accordingly, the written description would be sufficient for one of ordinary skill in the art to treat any autoimmune disease using any combination of IIC gp-39 fragments selected from one or more of SEQ ID NO: 1-8.

Feb. 20, 2004
Date

Andrea van Elsas
Name

REFERENCES

Joosten LAB, Coenen-de Roo CJJ, Helsen MMA, Lubberts E, Boots AMH, Van den Berg WB, Miltenburg AMM. Induction of tolerance with intranasal administration of Human Cartilage gp-39 in DBA/1 mice: Amelioration of clinical, histologic, and radiological signs of type II collagen-induced arthritis. Arthritis Rheum 2000; 43:645.

Wolvers DAW, Coenen-de Roo CJJ, Mebius RE, Van der Cammen MJF, Tirion F, Miltenburg AMM, Kraal G. Intranasally induced immunological tolerance is determined by characteristics of the draining lymph nodes: studies with OVA and Human Cartilage gp-39. J Immunol 1999; 162: 1994.

Baeten D, Boots AMH, Steenbakkers PGA, Elewaut D, Bos E, Verheijden GFM, Verbruggen G, Miltenburg AMM, Rijnders AWM, Veys EM, De Keyser F. Human Cartilage gp-39+, CD16+ monocytes in peripheral blood and synovium: Correlation with joint destruction in rheumatoid arthritis. Arthritis Rheum 2000; 43: 1233.

Krause SW, Rehli M, Kreutz M, Schwarzfischer L, Paulaskis JD, Andreesen R. Differential screening identifies genetic markers of monocyte to macrophage maturation. J Leukoc Biol 1996; 60: 540.

Rehli M, Niller H-H, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW. Transcriptional regulation of *CHI3L1*, a marker gene for late stages of macrophage differentiation. J Biol Chem 2003; 278: 44058.

Hakala BE, White C, Recklies AD. Human Cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. J Biol Chem 1993; 268: 25803.

Volck B, Price PA, Johansen JS, Sorensen O, Benfield TL, Nielsen HJ, Calafat J, Borregaard N. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. Proc Assoc Am Physicians 1998; 110: 351.

Johansen JS, Stoltenberg M, Hansen M, Florescu A, Horslev-Petersen K, Lorenzen I, Price PA. Serum YKL-40 concentrations in patients with rheumatoid arthritis: relation to disease activity. Rheumatology 1999; 38: 618.

Vos K, Steenbakkers P, Miltenburg AMM, Bos E, Van den Heuvel MW, Van Hogezand RA, De Vries RRP, Breedveld FC, Boots AMH. Raised Human Cartilage glycoprotein-39 plasma levels in patients with rheumatoid arthritis and other inflammatory conditions. Ann Rheum Dis 2000; 59: 544.

Boot RG, Van Achterberg TAE, Van Aken BE, Renkema II, Jacobs MJHM, Aerts JMFG, De Vries CJM. Strong induction of members of the chitinase family of proteins in atherosclerosis: Chitotriosidase and Human Cartilage gp-39 expressed in lesion macrophages. Arterioscler Thromb Vasc Biol 1999; 19: 687.

Kumar S, Bahn RS. Relative overexpression of macrophage-derived cytokines in orbital adipose tissue from patients with Graves' ophthalmopathy. J Clin Endocrin Metab 2003; 88: 4246.

D'Souza MJ, Oettinger CW, Shah A, Tipping PG, Huang XR, Milton GV. Macrophage depletion by albumin microencapsulated clodronate: attenuation of cytokine release in macrophage-dependent glomerulonephritis. Drug Dev Ind Pharm 1999; 25: 591.

Young L, Katrib A, Cuello C, Vollmer-Conna U, Bertouch JV, Roberts-Thomson PJ, Ahern MJ, Smith MD, Youssef PP. Effects of intraarticular glucocorticoids on macrophage infiltration and mediators of joint damage in osteoarthritis synovial membranes: findings in a double-blind, placebo-controlled study. Arthritis Rheum 2001; 44: 343.

Ramanan AV, Schneider R. Macrophage activation syndrome following initiation of etanercept in a child with systemic onset juvenile rheumatoid arthritis. J Rheumatol 2003; 30: 401.

Bredberg A, Henriksson G, Larsson A, Sallmyr A, Manthorpe R. A role of the macrophage in Sjögren's syndrome? Scand J Rheumatol 2003; 32: 255.

Shi FD, Bai XF, Li HL, Link H. Macrophage apoptosis in muscle tissue in experimental autoimmune myasthenia gravis. Muscle Nerve 1998; 21: 1071.

Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular cartilage damage in rheumatoid arthritis. Arthritis Rheum 1996; 39: 115.

Alric L, Pinelli E, Carrera G, Vinel JP, Beraud M, Duffaut M, Pascal JP, Pipy B. Involvement of calcium in macrophage leukotriene release during experimental cirrhosis. Hepatology 1996; 23: 614.

Pouvreau I, Zech JC, Thillaye-Goldenberg B, Naud MC, Van Rooijen N, De Kozak Y. Effect of macrophage depletion by liposomes containing dichloromethylene-diphosphonate on endotoxin-induced uveitis. J Neuroimmunol 1998; 86: 171.

Ren Y, Tang J, Mok MY, Chan AW, Wu A, Lau CS. Increased apoptotic neutrophils and macrophages and impaired macrophage phagocytic clearance of apoptotic neutrophils in systemic lupus erythematosus. Arthritis Rheum 2003; 48: 2888.

Klebl FH, Olsen JE, Jain S, Doe WF. Expression of macrophage-colony stimulating factor in normal and inflammatory bowel disease intestine. J Pathol 2001; 195: 609.

Bruck W, Porada P, Poser S, Rieckmann P, Hanefeld F, Kretzschmar HA, Lassmann H. Monocyte/macrophage differentiation in early multiple sclerosis. Ann Neurol 1995; 38: 788.

Kusterer K, Ludwig R, Hochschulz P, Stegmuller M, Bojunga J, Von Minckwitz G. Macrophage and lymphocyte homing in experimental diabetes. Exp Clin Endocrinol Diabetes 1999; 107 Suppl 3: S102.

Vos K, Miltenburg AMM, Van Meijgaarden KE, Van den Heuvel M, Elferink DG, Van Galen PJM, Van Hogezand RA, Van Vliet-Daskalopoulou E, Ottenhoff THM, Breedveld FC, Boots AMH, De Vries RRP. Cellular immune response to Human Cartilage glycoprotein-39 (HC gp-39)-derived peptides in rheumatoid arthritis and other inflammatory conditions. Rheumatology 2000; 39: 1326.